The Proteolytic Enzyme of Drosera.

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(Communicated by D. H. Scott, F.R.S., December, 1909, and subsequently withdrawn. Recommunicated September 6,—Read November 17, 1910.)

It has been known for many years that if pieces of raw meat be placed on the leaves of a living Drosera plant, the meat becomes decomposed, and the nutritious parts disappear, having been apparently absorbed by the plant. The decomposition was assumed to be brought about by means of proteolytic enzymes which were supposed to be present in the excretion of the glands with which the foliage leaves are abundantly supplied.*

As a general rule, some or all of the leaves of a plant growing under normal conditions are found to contain insects in various stages of decomposition, which have been caught and entrapped by the sensitive glandular hairs curling over their bodies and thus imprisoning them. It is found that the bodies of these insects undergo the same changes as the bits of raw meat did. The hairs do not unclasp the insect's body until all the nutritious matter which it contains has disappeared, and they only release their hold when the remaining parts are simply skeleton, wings, etc.

This decomposition of the body of the insect does not necessarily imply that proteolytic enzymes are excreted by the gland cells of the plant, for it is quite possible that the solution of the insect's body, and of the pieces of raw meat, may be accomplished by the action of putrefactive, or at least of enzyme-producing, bacteria. Tischutkin states that it is solely bacterial digestion which occurs in carnivorous plants.†

The same idea was put forward by Dubois‡ in his paper "Sur le prétendu pouvoir digestif du liquide de l'urne des Nepenthes."

The only record I could find of experiments performed for the purpose of precipitating the proteolytic enzymes, if actually present, in the glandular excretions of Drosera was that of Lawson Tait.§ In 1875 he isolated a proteolytic ferment from the glandular secretion of *Drosera dichotoma*, which he states closely resembles pepsin. The method he adopted for the preparation of the ferment was as follows:—

"The secretion from the Drosera was collected on a feather, which was

* 'The Soluble Ferments and Fermentation,' Reynolds Green, p. 214.

† 'Bot. Centralbl.,' 1892, vol. 50, p. 304, and 1893, vol. 53, p. 322.

‡ 'Compt. Rend.,' 1890, vol. 111, p. 315.

§ 'Nature,' 1875, vol. 12, p. 251.

then washed in pure distilled water. The solution was acidulated with dilute phosphoric acid, and then a thin mixture of chalk and water was added, drop by drop, until the effervescence ceased. The mixture was allowed to stand for 24 hours, when the clear fluid was removed.

"The precipitate was treated with very dilute hydrochloric acid, and the result treated with a saturated solution of pure cholesterin prepared by Bencke's method in a mixture of absolute alcohol and absolute ether. The mass which separated was then dissolved in absolute ether, and in the resulting water was suspended a greyish flocculent matter which was found to be perfectly amorphous. It was dried at a temperature of 42° C. When applied to a small quantity of fresh milk, the latter was observed to become coagulated."

This method appears to be exceedingly cumbersome, and the results to be unsatisfactory from the standpoint of proteolytic enzymes, as the ferment isolated was only noted to cause a viscid coagulation in milk. Besides, it is always possible that the cholesterin used might be contaminated with traces of various kinds of enzymes.

Vines* precipitated a proteolytic enzyme from the pitchers of Nepenthes in the following manner:—To a certain volume of liquid obtained from the pitchers an equal volume of absolute alcohol was added. To increase the bulk of this precipitate phosphoric acid and then lime water were added. Ammonium carbonate was added until the liquid became neutral, and the precipitate was filtered off. Some of the precipitate was shaken up with 0·2-per-cent. solution of HCl, and this liquid was again filtered, when the clear filtrate was found to actively digest fibrin.

In order to determine the true state of affairs concerning the proteolytic enzymes of Drosera, if any such do exist, I performed a series of experiments in which the enzymes were precipitated from the fresh leaves of the following species of Drosera:—*D. auriculata, D. Menziesii, D. peltata, D. Whittakeri.*

The method adopted for their precipitation was practically the same as was employed in connection with an investigation of the enzymes and latent life of seeds.† The leaves were cut off from the plants, and all traces of foreign matter were removed from them. They were then washed in cold boiled water, and again in a strong solution of chloroform, which acts as an antiseptic; in this solution they became quite flaccid. The leaves were now chopped up into minute pieces with a sterilised knife, and the fragments weighed and put into a bottle containing about 100 c.c. of lukewarm boiled

* 'Ann. Bot.,' 1897, vol. 11, p. 573.

† 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 424.

water and about 30 drops of chloroform. The bottle was shaken vigorously for two hours, when its contents were filtered.

To the filtrate was added half its volume of saturated solution of ammonium sulphate, which was found to be far more efficient in this instance for precipitating the proteins and enzymes than methylated spirit. The precipitate produced in this way was filtered off on to filter paper which had been previously sterilised with boiling water, and was dried in a sulphuric acid desiccator. When almost dry, the precipitate was scraped off the filter paper with a sterilised knife and dissolved in cold boiled water.

A small quantity of this solution was put into four test-tubes, A, B, C, and D. The contents of A were boiled, and into A and B was put a little well washed and teased fibrin. The contents of C were also boiled, and into C and D was put a small amount of Witte peptone. About 20 drops of chloroform were poured into each test-tube, and each tube was carefully shaken up. The ends were plugged with cotton wool, and the tubes placed in an oven at 35° C. Samples of the freshly made solution were tested for the biuret and tryptophane reactions, and it was found that distinct traces of peptone were discernible, but there was no sign of amido bodies in the solution.

After a period of time varying from about 30 to 50 hours, the test-tubes A, B, C, and D were removed from the oven, and their contents were tested for evidences of the activity of proteolytic enzymes. Application of the biuret test to tubes A and B disclosed the presence of a considerable quantity of peptone in B, while traces were detected in A in about the same proportion as existed in the freshly made solution.

To tubes C and D, the tryptophane test was applied, with the result that there were not the minutest signs of the presence of any amido bodies.

Results of Experiments.

Species.	Time of digestion.	Reaction to biuret.	Reaction to tryptophane.
D. auriculata...........	50 hours	Good reaction	No reaction
D. Menziesii	40 "	"	"
D. peltata	40 "	"	"
D. Whittakeri	40 "	Very good reaction	"

The results of this series of experiments, most of which were performed several times with different specimens of the same species of Drosera, serve

to indicate that apparently peptone is the ultimate product of the digestion of proteins by the digestive fluids of Drosera. It also shows that peptones are present in small amounts in the extract prepared from the leaves of the different species of Drosera, even in the ammonium sulphate precipitate, and that the amount is very rapidly increased if the extract is left at a fairly high temperature in contact with fibrin.

This latter fact points to the existence of a pepsin-like enzyme which is apparently present in considerable quantities, and is not in the form of a zymogen. This latter statement agrees with that of Vines, who in his previously mentioned paper on the proteolytic enzymes of Nepenthes, came to the conclusion that the pitcher liquid of Nepenthes contains no zymogen.

As the digestive process does not appear to extend beyond the production of peptones, it seems as if no traces of other proteolytic enzymes such as erepsin or trypsin are present; this does not altogether coincide with the results obtained by Vines in his researches on the pitcher liquid of Nepenthes.

In accordance with his results, I have made very many attempts to find even the minutest signs of protein digestion carried to the amide stage, but have always been unsuccessful. I have been therefore forced to the conclusion of the non-production of these bodies in the species of Drosera mentioned, when growing under their normal conditions in the Victorian bush. It is in fact hardly to be expected that all carnivorous plants should carry out their digestion in exactly the same way and by the same means.

In order to ascertain whether the leaves were capable of absorbing peptones, I performed the following simple experiment.

A saturated peptone solution was prepared with carefully sterilised water, and after filtering it, two drops of the clear solution were applied to five similar marked leaves (which were apparently perfectly horizontal) of plants of *Drosera Whittakeri* which were growing in a flower-pot. One of these leaves was cut off at once, and also a similar leaf to which no peptone solution had been added. The pot was now covered over with a large bell jar, the air inside which was kept damp.

After leaving the flower-pot undisturbed for five hours the marked leaves were cut off and put into sterilised water and the biuret test applied. But in no case was any peptone reaction discernible, thus showing that, while all the water from the solution placed on the leaves had not completely disappeared, the peptone dissolved in the solution had done so, having been absorbed by the leaves of the plant.

Careful microscopic examination was made of the leaves after being cleaned, and also of those in which the remains of the insects' bodies were still

present, but no signs of bacteria were visible, so that it seems most probable that the digestive fluid exerts an antiseptic action.

Since the above was written, Prof. Vines was so good as to give me the benefit of certain suggestions and criticisms during a visit made to England, and these were such as to render it advisable to repeat certain of the experiments under different conditions and with varying controls. For this purpose *Drosera Whittakeri* was selected.

Prof. Vines suggested that the strong chloroform used as an antiseptic might exercise an inhibitory effect on the enzyme action. To ascertain if this were the case, the experiments were repeated just as before, with the exception that 4 drops of 0·1-per-cent. HCN were added to each 100 c.c. of the digestive solution in the test-tubes, in place of the chloroform previously employed as an antiseptic. After leaving the test-tubes for 40 hours in an oven at 32° C. I found that the same results were arrived at as before, *i.e.* that marked fibrin digestion had occurred, but no trace of any digestion of peptones could be detected.

Prof. Vines further mentioned that the reaction of the digestive solution might affect the activity of the enzymes present in it. In order to test this point, the second additional series of experiments was performed. The freshly made digestive solution was found to be neutral, and into each of 12 test-tubes (A—L) was poured 100 c.c. of this neutral solution, and four drops of 0·1 per cent. hydrocyanic acid. The contents of each test-tube were then treated as follows:—

The contents of test-tubes A, B, C, D, were left neutral. The solution in B and D was boiled. To A and B was added a little well washed fibrin. To C and D a small quantity of Witte peptone was added. The contents of E, F, G, and H were acidified by the addition of a few drops of 0·2-per-cent. hydrochloric acid. The solution in F and H was boiled. To E and F fibrin, to G and H Witte peptone was added. The contents of I, J, K, L were rendered alkaline by pouring into each test-tube a few drops of a 1-per-cent. solution of sodium carbonate. The solution in J and L was boiled. To I and J fibrin, to K and L Witte peptone was added.

The test-tubes were then plugged with sterilized cotton wool and put into the oven at 32° C. as before. After 40 hours the contents of all the test-tubes were tested and it was found that A, E, and I all gave a strong peptone reaction, the strength of which was apparently the same in each; B, F, and J gave a faint peptone reaction of the same strength as the original freshly made solution exhibited, showing that in these boiled samples, no further protein digestion had taken place.

On application of the tryptophane test to the contents of the test-tubes C, D, G, H, K, and I, it was found that in none of the tubes could there be detected the faintest trace of amido bodies or of any digestion carried beyond the peptone stage, and the same result was obtained when the tubes had been put back into the oven for an additional 24 hours.

The results of these additional experiments must consequently serve to strengthen my previous conclusion that the digestive process by the proteolytic enzyme present in the leaf glands of Drosera does not extend further than the production of peptones.

In conclusion I wish to thank Prof. Ewart for all the help he has given me. Supplies of fresh material of the different species tested were obtained as they were required by Mr. J. W. Audas, of the National Herbarium.

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